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## Inhibition of glucose-6-phosphate dehydrogenase from Ehrlich ascites carcinoma by ATP

ATP has been shown to inhibit the glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate:$NADP^+$ oxidoreductase, EC 1.1.1.49) from both yeast[1-4] and brain[3]. This inhibition apparently arises from a competition with glucose 6-phosphate[1] and $NADP^+$ (refs. 2, 3) for the active sites and from a separate allosteric inhibition by ATP[2]. The inhibitory effects of ATP are diminished by the presence of $Mg^{2+}$ (refs. 1, 3, 4). The work described below extends the finding of an ATP inhibition to the dehydrogenase from Ehrlich ascites tumor cells and indicates that glucose 6-phosphate as well as ATP may be involved in an allosteric inhibition of the enzyme.

*Preparation of extract.* A hyperdiploid strain of Ehrlich ascites carcinoma was grown for 7 days in Swiss White or Strong A mice, harvested, and washed in a medium containing 100 mM KCl, 48 mM NaCl, 2.25 mM $MgCl_2$, 5 mM Tricine buffer (pH 7.3) and 2 g/100 ml bovine serum albumin. Packed cell volume was determined on an aliquot of the final suspension[5], and the bulk of the suspension, about 30 ml containing 20–40% (v/v) cells, was exposed to 820 lb/inch$^2$ $N_2$ in an Artesan Industries pressure homogenizer (as described in refs. 6 and 7) for 20 min and then suddenly released to atmospheric pressure through a narrow aperture to rupture the cells. Nuclei and cellular debris were removed by centrifugation at 1500 $\times g$ for 15 min, and the supernatant was used directly in the assays for enzyme activity. Other studies have indicated that the glucose-6-phosphate dehydrogenase activity is entirely contained in the 150 000 $\times g$ supernatant (W. V. V. GREENHOUSE, unpublished observations).

*Assay of enzyme activity.* Reaction mixtures containing 1.0 ml 0.10 M Tricine buffer (pH 7.5), 0.10 ml 2.5 mg/ml (approx. 3 mM) $NADP^+$, and standard solutions of glucose 6-phosphate to give final concentrations of 0.05–10.0 mM and ATP to give final concentrations of 0.0–4.0 mM were made up to 2.60 ml in 3-ml cuvettes with a 1.0-cm light path. The reaction was followed by observing the extent of reduction of the $NADP^+$ at 340 nm with time in a Beckman model DU spectrophotometer equipped with 38° thermospacers, ERA adapter, and Sargeant model SRLG recorder set for linear absorption reading. Cuvettes were pre-warmed to approx. 38°, and the reaction was initiated by addition of 0.05 ml tumor cell extract. The velocity was estimated from the initial slopes of the recorded curves. These velocities were converted to $\mu$moles/ml packed cells per min, using the extinction coefficient of NADPH, the determined packed cell volume, and the appropriate dilution factor. When compared on the basis of packed cell volumes, the velocities were highly consistent from one preparation to another; for example, in 5 different preparations at 0.5 mM glucose 6-phosphate and no ATP, the velocities averaged 3.60 and ranged from 3.24 to 3.95 $\mu$moles/ml cells per min.

*Results.* Fig. 1 presents the velocity as a function of glucose 6-phosphate concentration up to 1.5 mM in 0.0, 2.0, and 4.0 mM ATP. Superficially, the curves appear to follow the expected Michaelis–Menten relationship, the half maximal velocity occurring at 0.03–0.04 mM glucose 6-phosphate at 0 mM ATP. Since the intracellular ATP concentration ranges from 2 to 4 mM and glucose 6-phosphate from 0.1 to 0.5 mM[8,9] in these cells, the ATP inhibition evident in Fig. 1 could play a physiological role. It

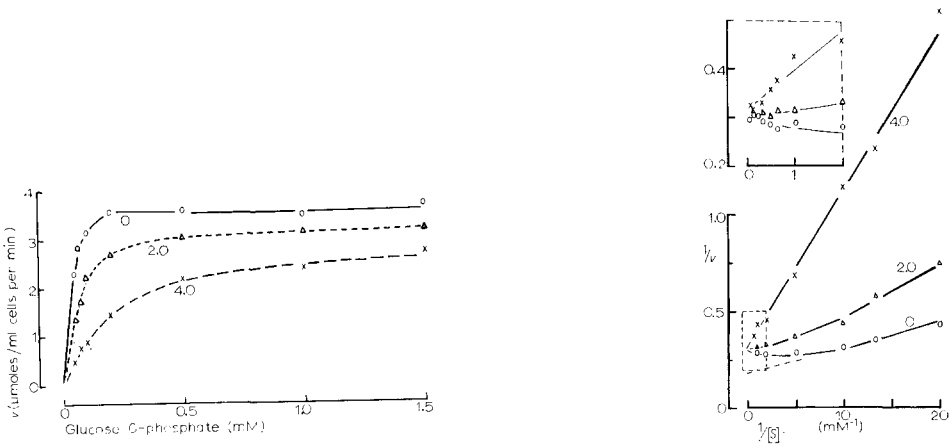


Fig. 1. Velocity of $NADP^+$ reduction as a function of glucose 6-phosphate concentration. The reaction mixture contained 38 mM Tricine buffer (pH 7.5), 0.1 mM $NADP^+$, 0.05 ml tumor extract and the indicated concentrations of glucose 6-phosphate and ATP at 37° (see text). ○—○, rate in absence of ATP; △- - -△, in 2.0 mM ATP; ×— —×, in 4.0 mM ATP.

Fig. 2. Double reciprocal plot of velocity *versus* substrate concentration. [$S$] = glucose 6-phosphate concentration. ○—○, in absence of ATP; △—△, in 2.0 mM ATP; ×—×, in 4.0 mM ATP. Inset at upper left shows boxed region around low $1/[S]$ values on an expanded scale. Values for 0 mM ATP represent averages of from 3 to 5 preparations; values for 2.0 and 4.0 mM ATP are averages from 2 or 3 preparations.

should be noted, however, that these curves were obtained at a low $Mg^{2+}$ concentration (approx. 0.04 mM final concentration in the assay mixture, contributed by the cell extract) and that the intracellular levels of $Mg^{2+}$ (10 mM or more) may be sufficient to reverse much of the ATP inhibition[1,3,4].

Substantial deviations from the simple Michaelis–Menten relationship are evident when a double reciprocal plot of velocity and glucose 6-phosphate concentration is examined (Fig. 2). Only the enzyme inhibited by 4.0 mM ATP approximates the expected linear relationship; the others show a distinct upward curvature as $1/[S]$ approaches zero. The inset in Fig. 2, giving the low values of $1/[S]$ on an expanded scale, illustrates that all three curves converge toward the same velocity at high glucose 6-phosphate concentrations. The curvature in Fig. 2 would imply a decrease in $v$ at high concentrations in Fig. 1, although this is not apparent because the higher concentrations of glucose 6-phosphate which show a definite decrease are beyond the range covered by this figure. Linear extrapolation of the low $1/[S]$ values at 0.0 mM ATP in Fig. 2 indicates a $v_{max}$ of about 5.4 $\mu$moles/ml cells per min and a $K_m$ of 0.07 mM. Since the ATP inhibition is not a simple competitive type, a $K_i$ value cannot be calculated.

These results differ from those of AVIGAD[1], who found a simple competitive inhibition by ATP for the glucose 6-phosphate site on yeast glucose-6-phosphate dehydrogenase and agree with the finding of BONSIGNORE *et al.*[2] that an allosteric as well as a competitive inhibition may be involved. In the absence of $Mg^{2+}$, the $K_m$ for yeast glucose 6-phosphate is about 0.04 mM[1,2], whereas in 5 mM $MgSO_4$ it is increased to 0.07 mM[1]. ATP appears to have a stronger effect on the yeast enzyme, since 2.0 mM ATP causes a 70% inhibition at 0.05 mM glucose 6-phosphate in the absence of $Mg^{2+}$

(ref. 1), whereas it causes only about 40% inhibition with the tumor enzyme under comparable conditions. It should be noted, however, that the tumor "enzyme" was actually a crude extract and probably contained 6-phosphogluconate dehydrogenase (EC 1.1.1.44). Although initial rates of $NADP^+$ reduction were used to minimize the possible contribution of this enzyme, some contribution cannot be completely excluded.

The relationship between velocity and substrate concentration seen in Fig. 2 could be explained on the basis of two assumptions: (a) there are two different types of sites on the enzyme, the active sites and separate inhibitory sites which modify the properties of the active sites, as proposed by BONSIGNORE *et al.*[2]; and (b) both types of sites are capable of binding either glucose 6-phosphate or ATP. At high concentrations of ATP, the inhibitory sites would be permanently saturated with ATP or a combination of ATP and glucose 6-phosphate, and linear double reciprocal plots reflecting a simple competitive inhibition at the active site would be obtained. At low ATP concentrations, the inhibitory sites would be largely unsaturated until higher levels of glucose 6-phosphate were attained, and the double reciprocal plot would consequently show an upward curvature at low values of $1/[S]$ and approach the same intercept on the $1/v$ axis as the enzyme in the presence of a high ATP concentration. Deductions more elaborate than this should be postponed until information from a purified ascites tumor dehydrogenase is available.

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